ELISA Kit Components	Amount	Cat/Part No.
Anti-Mouse TNF Microwell Plate	8-well strips (12)	100-211
Mouse TNF Positive Control	0.65 ml	100-212
Mouse TNF Standard 50 pg/ml	0.65 ml	100-213B
Mouse TNF Standard 100 pg/ml	0.65 ml	100-213C
Mouse TNF Standard 250 pg/ml	0.65 ml	100-213D
Mouse TNF Standard 500 pg/ml	0.65 ml	100-213E
Mouse TNF Standard 1000 pg/ml	0.65 ml	100-213F
Anti-Mouse TNF Detection Ab (100X)	0.15 ml	100-214
Streptavidin HRP Conjugate (100X)	0.15 ml	S-HRP100
Sample Diluent Concentrate (20X)	10 ml	SD-20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-100-210-TNM

Other ELISA kits available from ADI

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG3, IgG2b, IgM, Leptin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF, SAP.

Human: BD-1, BD-2, BD-3, NP-1 **and**: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, Angiogenin, Angiopoietin-2, beta-2M, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgA, Insulin, NSE, CA125, CA199, CA242, PAP, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Monkey: IgM, IgG, IgA, CRP, IgE
Chicken: IgY(G), IgM, Ovalbumin
Rabbit: CRP, IgG
Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM
Cat: IgG, IgM
Goat: IgG

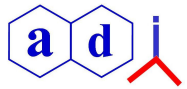
For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. M-100-210-TNM

Mouse TNFα

ELISA Kit Cat. No. 100-210-TNM

For Quantitative Determination of Mouse Tumor Necrosis Factor Alpha in Solution



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INTENDED USE

The Mouse TNFα ELISA Kit is an in vitro immunoassay for research use in the quantification of Mouse TNFα in cultures of mouse cells and in appropriately qualified samples from serum, saliva, or other tissue fluids.

INTRODUCTION

Tumor Necrosis Factor, or TNF alpha, is a key inflammatory cytokine and the prototype of a large family of secreted and membrane-bound cytokines that regulate both innate and adaptive immune responses and developmental events. TNFα is a homotrimer, and is cleaved from the membrane to release a soluble regulator that acts locally but can have systemic effects.

TNFα acts in concert with the superfamily of TNF-receptors to promote and/or inhibit inflammation, both early when it is produced by innate immune cells including macrophages, and later, when it is produced by T lymphocytes. Thus, TNFα is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation, and has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Knockout studies in mice also suggested the neuro-protective function of this cytokine. The induction of inflammation by TNFα, with the recruitment of immune cells to sites of infection, operates as a transient and reversible organization of tissue to foster interaction between immune cells; this is a common attribute of members of the TNF superfamily. The inhibition of TNFα's action has been a pharmacological strategy for the suppression of inflammation in a variety of diseases.

PRINCIPLE OF THE TEST

The Mouse TNFα ELISA kit is based on the binding of Mouse TNFα in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to biotin, which then binds to a streptavidin horseradish peroxidase (HRP) conjugate. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of TNFα present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of TNFα in samples is calculated from a standard curve of purified recombinant mouse TNFα of designated concentration.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

Specificity
The antibodies used in this kit have been affinity purified using a purified recombinant mouse TNFα immunosorbent and have been shown by ELISA to react specifically with mouse and rat TNFα, and to have essentially no reactivity with the following proteins: mouse IL-2 and CD40L; human TNFα.

Precision
Samples containing low, medium and high concentrations of TNFα were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations were calculated for the concentrations using a point-to-point curve-fitting program.

TNFα concentrations were measured with good within-assay (4.5 to 6.9 %CV) and between-assay (3.1 to 6.7 %CV) reproducibility.

Sample	TNFα pg/ml	Intra-assay %CV	Inter-assay %CV
Low Concentration	188	nd	**6.7**
Medium Concentration	339	**4.5**	**3.1**
High Concentration	488	**6.9**	**4.7**

Mouse Serum
TNFα Levels
Assay of stored sera from twenty (20) individual adult mice showed no measurable TNFα at 1:5 dilution. Each laboratory should determine expected values of its own testing population.

Recovery
Purified TNFα was spiked into each of 6 stored serum samples, non-diluted. Observed assay values compared to expected values ranged from 37 to 72%. The suppression of recovery was essentially alleviated by diluting 1:5 or greater.

Culture Medium
Linearity of Dilution and Recovery
TNFα was spiked into 10% Neonatal Bovine Serum in Sample Diluent at 3 levels, 100-500 pg/ml. The mean recovery ranged from 69 to 82%, demonstrating incomplete recovery and non-linear dilution curve. Recovery and linearity were restored with 1:5 dilution of the samples. If culture media samples are required to be tested undiluted, for accurate quantitation prepare the standards as 1:5 dilutions in the control culture medium.

CALCULATION OF RESULTS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Mouse TNFα concentrations may be determined as follows:

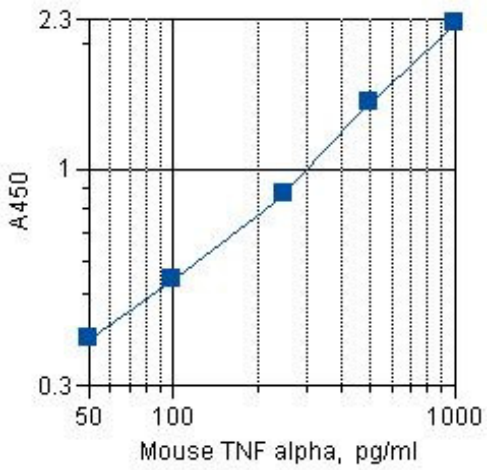
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (pg/ml) of Mouse TNFα (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The Mouse TNFα concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 1000 pg/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm mean	TNFα pg/ml
1A, B	**Negative Diluent Control**	0.21	0
1C, D	50 pg/ml **Standard**	0.39	50
1E, F	100 pg/ml **Standard**	0.54	100
1G, H	250 pg/ml **Standard**	0.87	250
2A, B	500 pg/ml **Standard**	1.45	500
2C, D	1000 pg/ml **Standard**	2.25	1000
2G, H	**Positive Serum Control** [Value: 502 – 934 pg/ml]	1.03	304
3A, B	**Sample** [Diluted 1:5]	0.90	261
	Calculated: 5-fold dilution x 261 pg/ml = **1.30** ng/ml in medium		

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

Ready For Use: Store as indicated on labels.

Component	Part #	Amt	Contents
Anti-Mouse TNFα Microwell Strip Plate	100-211	8-well strips (12)	Coated with purified anti-Mouse TNFα antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
Positive Control [TNFα] range on label	100-212	0.65 ml	TNFα with stated concentration range; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
Mouse TNFα Standards			
50 pg/ml 100 pg/ml 250 pg/ml 500 pg/ml 1000 pg/ml	100-213B 100-213C 100-213D 100-213E 100-213F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing the specified concentration of rTNFα ; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Anti-Mouse TNFα Detection Antibody Concentrate (100x) Part No. 100-214, 0.15ml	Biotinylated anti-Mouse TNFα in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.
Streptavidin-HRP Conjugate Concentrate (100x) Part No. S-HRP100, 0.15ml	Peroxidase conjugated streptavidin antibody in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipetter is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples, Detection Antibody Concentrate and Streptavidin-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and Detection Antibody contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference.

For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For **other samples**, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately , stored refrigerated for up to a week, or frozen for long-term storage. Avoid freeze-thaw cycles.

QUALITY CONTROL

Sample Controls A Positive Serum Control is provided with the kit, assigned with a TNFα concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer may be used. ELISA reader and pipettes should be properly calibrated.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Samples at least 5-fold in Working Sample Diluent, e.g., 100ul sample + 400ul Diluent. See Mouse Serum and Culture Medium under Performance Characteristics.
DO NOT dilute the Standards or Positive Control Serum.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, to include 12 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand about 5 minutes before sample addition.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

2. 1st Incubation [100ul - 90min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. An automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul - 60min; 3 washes]

- Add 100ul of Working Detection Antibody to each well.
- Incubate for 60 minutes.
- Wash wells 3 times as in step 2.

4. 3rd Incubation [100ul - 30min; 5 washes]

- Add 100ul of Working Streptavidin-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

5. Substrate Incubation [100ul - 15min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

6. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

7. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.